

PCT/US00/06560

-18-

That which is claimed is:

- A method of producing full-length coding sequences, said method comprising:
 - (a) synthesizing first strand cDNA using isolated full-length mRNA from a population of cells as a template, thereby forming first strand cDNA/mRNA hybrid(s);
 - (b) denaturing the first strand cDNA/mRNA hybrid(s);
 - (c)\attaching a non-native tag sequence to the 3' end of the first strand cDNA; and
 - (d) producing a full-length double-stranded cDNA by synthesizing second strand cDNA using the tagged first strand cDNA produced in step (c).
- 2. A method according to claim 1 wherein the mRNA is isolated employing an affinity purification material.
- A method according to claim 2 wherein the affinity purification 3. material comprises one or more cap-binding proteins bound to a solid surface.
- A method according to claim 3 wherein the cap-binding protein(s) are 4. selected from the group consisting of eIF4E, eIF4G, eIF4F, eIF4G, nCBP, and eIF4E:eIF4G fusion protein.
- 5. A method according to claim 2 wherein the mRNA to be isolated comprises a biotinylated cap structure.
- 6. A method according to claim 5 wherein the affinity purification material is a streptavidin or avidin-complexed solid support.
- 7. A method according to claim 1 wherein the mRNA is de-capped and de-phosphorylated after isolation.

PCT/US00/06560



8. A method according to claim 1 wherein the tag sequence comprises a recognition site for a site-specific recombinase.

-19-

- 9. A method according to claim 8 wherein the tag sequence further comprises a recognition site for a site-specific restriction endonuclease.
- 10. A method according to claim 1 wherein the tag sequence is attached by a site-specific recombinase capable of recognizing and acting on single stranded DNA.
- 11. A method according to claim 10 wherein the site-specific recombinase is *E. coli* topoisomerase III.
- 12. A method according to claim 1 further comprising amplifying the cDNA during or after step (d).
- 13. A method according to claim 12 further comprising inserting the amplified cDNA into an expression vector.
- 14. A method according to claim 1 further comprising treating the first strand cDNA/mRNA hybrid(s) formed in step (a) with a substance that degrades single stranded RNA; and isolating the undegraded hybrid(s) with an affinity purification material having affinity for capped mRNA prior to performing step (b).
 - 15. A method according to claim 14 wherein the substance is RNAse I.
- 16. A method according to claim 14 wherein the affinity purification material comprises one or more cap-binding proteins bound to a solid support.
- 17. A method according to claim 14 wherein the mRNA component of the cDNA/mRNA hybrid comprises a biotinylated cap structure.

SUB B' COO.t.

-20-

- 18. A method according to claim 17 wherein the affinity purification material is a streptavidin or avidin-complexed solid support.
- 19. A method according to claim 14 further comprising inserting the double stranded cDNA resulting from step (d) into an expression vector.
- 20. An isolated full-length coding sequence prepared according to the method of claim 1.
- 21. An expression vector comprising an isolated full-length coding sequence prepared according to the method of claim 1.
- 22. An expression vector according to claim 21 comprising one or more elements selected from: a promoter-enhancer, a selection marker encoding sequence, an origin of replication, an epitope tag encoding sequence or an affinity purification-tag encoding sequence.
- 23. An expression vector according to claim 22 wherein the promoter-enhancer is the T7 promoter, gall promoter metallothionein promoter, AraC promoter, or CMV promoter-enhancer.
- 24. An expression vector according to claim 22 wherein the selection marker encoding sequence encodes a protein which imparts antibiotic resistance to cells.
- 25. An expression vector according to claim 22 wherein the epitope-tag sequence encodes V5, the peptide Phe-His-His-Thr-Thr (SEQ ID NO:2), hemaglutinin, or glutathione-S-transferase.
- 26. An expression vector according to claim 22 wherein the affinity purification-tag sequence encodes a polyamino acid tag or a polypeptide.

SUB) BI WO 00/56878 PCT/US00/06560

-21-

- 27. An expression vector according to claim 26 wherein said polyamino acid tag is polyhistidine.
- 28. An expression vector according to claim 26 wherein said polypeptide is a chitin binding domain or glutathione-S-transferase.
- 29. An expression vector according to claim 26 wherein said polypeptide encoding sequence includes an intein encoding sequence.
- 30. An expression vector according to claim 21 wherein the expression vector is a eukaryotic expression vector or a prokaryotic expression vector.
- 31. An expression vector according to claim 30 wherein the eukaryotic expression vector is pYES2, pMT, pIND, or pcDNA3.1.
 - 32. A method of obtaining full length coding sequences comprising:
 - (a) contacting full-length mRNA, isolated from a population of cells by employing an affinity purification material, with reverse transcriptase, thereby synthesizing first strand cDNA and forming first strand cDNA/mRNA hybrids;
 - (b) treating the first strand cDNA/mRNA hybrids with a substance that degrades single stranded RNA;
 - (c) isolating undegraded hybrid(s) from degraded hybrids employing an affinity purification material having affinity for capped mRNA;
 - (d) denaturing the isolated cDNA/mRNA hybrids obtained from step (c) thereby producing single stranded cDNA and single stranded mRNA;
 - (e) attaching a non-native tag sequence to the single-stranded cDNA, wherein the tag sequence comprises a site-specific recombination sequence and is attached by *E. coli* topoisomerase III; and
 - (f) synthesizing second strand cDNA using the tagged cDNA as a template and/or amplifying the cDNA, wherein the amplification primers comprise an anti-coding sequence of the tag sequence (5') and oligo-dT (3').

SUB B1





-22-

SUB CODITI

33. A method according to claim 32 further comprising inserting the cDNA obtained in step (f) into and expression vector.

34. A fusion protein comprising eIF4E and eIF4G.